

REMARKS

The present invention relates to a method of inducing and enhancing the proliferation of human bone marrow stromal cells.

Claim Amendments

Claim 24 has been amended herein to correct a typographical error in the claim as submitted in the Amendment filed on December 22, 2004. The article “a” should have been “at”. The present amendment adds no new matter, but rather corrects a typographical error.

Rejection of Claims 1-12, 14-29 and 31-36 Pursuant to 35 U.S.C. § 102(b)

Claims 1-12, 14-29 and 31-36 stand rejected pursuant to 35 U.S.C. §102(b) as allegedly being anticipated by Huang et al. (1997, Biotechnology Letters, 19: 89-92, hereinafter “Huang”). The Examiner contends that Huang teaches a method of propagating marrow stromal cells at low density that has the same steps as the presently claimed method. The Examiner asserts that the source of the cells (e.g. mouse versus human) does not materially change the steps of the method. Further, in the Office Action dated August 10, 2004, on which the Examiner also relies for the present rejection, the Examiner asserts that reciting a replating step is inherently present in cell culture methods known in the art.

MPEP § 2131 provides: “A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” Huang does not teach each and every element of claims 1-12, 14-29 and 31-36, and therefore does not anticipate these claims.

Huang does not teach a method for inducing proliferation of marrow stromal cells by plating the marrow stromal cells at an initial density of less than about 50 cells per cm², replating the marrow stromal cells at an initial density of less than about 50 cells per cm², and expanding the cells by at least ten fold, as set forth in claim 1. Huang also fails to teach the conditions for inducing proliferation of marrow stromal cells recited in dependent claims 2-23, and the methods recited in claims 24-29 and 31-36. Huang fails to teach each and every element of these claims because Huang does not teach at least two of the elements recited in the claims,

namely that marrow stromal cells are 1) replated to induce 2) at least 10-fold proliferation. Thus Huang cannot anticipate the claim.

The Examiner contends, in the Office Action dated August 10, 2004, that replating the cells is inherently present in cell culture methods, and as such does not recite any specific embodiment that would make it distinct from the prior art of record. The Examiner cites reasons for replating cells, such as maintaining cells in culture, preparing new cell lines, starting new cultures from a source, or cloning a single cell. None of these supposedly inherent reasons for replating cells recited by the Examiner is for inducing proliferation of a cell, as recited in the claims, and therefore replating cells is not inherently present in a method for inducing proliferation of cells. MPEP § 2112 provides:

“In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristics necessarily flows from the teachings of the prior art. (emphasis in original)

The Examiner has failed to meet the burden established by the MPEP. Huang does not teach that replating cells necessarily flows from plating cells at low density. Huang teaches that only one out of three cell lines plated at low densities even results in cell growth. The one cell line that does grow (LC3), grows poorly at the initial plating densities recited in the claims, and Huang provides no teaching, no suggestion, not even a hint, that replating these cells will result in proliferation. In fact, Huang does not teach replating the cells, only counting them, and the present claims do not recite counting the cells. Therefore, there exists no fact and/or technical reasoning in Huang to support the Examiner’s contention that replating marrow stromal cells is inherent.

Beyond Huang, there is no basis in fact and/or technical reasoning cited by the Examiner to support the contention that replating the cells is inherent. As noted above, the Examiner cites maintaining cells in culture, preparing new cell lines, starting new cultures from a source, or cloning a single cell as inherent reasons for replating cells. Maintaining is not inducing proliferation, preparing new cell lines is not inducing proliferation, starting new cultures from a source is not inducing proliferation, and cloning a single cell is not inducing proliferation. The reasons for replating cited by the Examiner are not relevant to the presently

claimed subject matter, and broad speculation as to the multitude of reasons one of skill in the art might want to replate a population of cells is not embodied in the present claims. The Examiner must demonstrate a basis in fact and/or technical reasoning for replating cells that supports the inherent characteristics necessarily flowing from Huang. Huang fails to teach or suggest replating, and none of the reasons for replating cited by the Examiner are relevant to the scope of the claims, which recite methods for inducing proliferation. Therefore, replating marrow stromal cells is not inherent and Huang cannot anticipate the present invention.

Even if replating marrow stromal cells is inherent, Huang still fails to teach each and every element of the claimed invention. Specifically, Huang fails to teach that plating cells at a low density or replating the same cells at a low density allows the cells to expand by a factor of at least 10-fold. Huang teaches that five LC1 or five LC2 cells plated on a 1.77 cm² culture dish results in zero growth, which is not a factor of 10-fold. Huang teaches that 50 LC1 or 50 LC2 cells plated on a 1.77 cm² culture dish results in zero growth, which is not a factor of 10-fold. Huang teaches that five LC3 or 50 LC3 cells plated on a 1.77 cm² culture dish results in the formation of one colony or four colonies, respectively. How many cells are in a colony? Huang does not teach how many cells are in a colony. It is impossible to determine if each and every element of the claims is recited in Huang because Huang does not teach if a single plating, let alone a replating, results in the marrow stromal cells expanding by a factor of at least 10-fold.

If a colony is one cell, Huang does not teach each and every element of the invention because plating 5 cells results in one cell, and plating 50 cells results in four cells. If a colony is 10 cells, Huang does not teach each and every element of the invention because plating 5 cells results in ten cells, and plating 50 cells results in forty cells. Applicants could continue to speculate as to the fold-increase in cells taught in Huang, but it is a fruitless effort because Huang does not teach how many cells are in a colony, and therefore, it is impossible to determine if Huang teaches each and every element of the claims. Without teaching each and every element of the claims, Huang cannot anticipate the present invention. Further, not only does Huang fail to teach a 10-fold increase in the number of cells from an initial density, Huang fails to teach a 10-fold increase in cells from a replated density, which is recited by the claims. Again, Huang fails to anticipate the present invention.

For the reasons stated above, Applicants respectfully submit that Huang fails to

teach each and every element of the recited claims, as is required by law, and therefore fails to anticipate the present invention. Reconsideration and withdrawal of the rejection of claims 1-12, 14-29 and 31-36 is respectfully requested at this time.

Rejection of Claims 1-12, 14-29 and 31-36 Pursuant to 35 U.S.C. §103(a)

Claims 1-12, 14-29 and 31-36 stand rejected pursuant to 35 U.S.C. §103(a) as allegedly being rendered obvious by Huang. The Examiner contends in this Office Action and the Office Action dated August 10, 2004, that the present claims are rendered obvious for the reasons discussed above for the anticipation rejection.

In order to establish a case of *prima facie* obviousness, the Examiner bears the burden of demonstrating the following three criteria: that there is some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP § 2142.

Huang provides no suggestion or motivation, whether alone or combined with the knowledge of one of skill in the art, to induce proliferation of marrow stromal cells by plating the cells at low density and replating the cells at a second low density such that the cells are expanded by at least 10-fold. Huang teaches initially plating the cells at a low density, but does not teach replating the cells at any density. Huang teaches counting the cells. This is not replating. The suggestion or motivation could not come the knowledge generally known in the art because the skilled artisan, when armed with Huang's disclosure, is aware that in two out of three instances (cell lines LC1 and LC2), plating the cells at low density, the density claimed in the present application, results in no growth whatsoever. Unless the Examiner is aware of some knowledge generally available to the skilled artisan teaching that replating cells that fail to grow results in an expansion of at least 10-fold, the Examiner has not met the burden required by law.

Even in the case of the LC3 cell line, which did develop colonies when plated at the initial low density recited in the present claims, Huang does not teach or suggest that replating these cells will result in an expansion of at least 10-fold. Huang teaches that plating these cells once results in the formation of one colony from five cells and four colonies from 50

cells. Not only is it speculation to assume that the number of cells produced are a 10-fold expansion, because Huang does not teach how many cells are in a colony, the colonies reported are the result of an initial plating, not replating, and Huang provides no teaching or suggestion that replating the cells would result in a 10-fold expansion of those cells.

In order to establish a *prima facie* case of obviousness the onus is on the Examiner to establish a reasonable expectation of success. To the best of Applicants' knowledge, "reasonable expectation" is not a quantifiable measure. However, there is no reasonable expectation if three cell lines (LC1, LC2 and LC3) are plated at the initial densities presently claimed, and two of the cell lines (LC1 and LC2) fail to grow at all, and then the experiment is repeated, and the same two cell lines again fail to grow. If this 2-fold failure to grow in 66.7% of the cell lines is coupled with the fact that Huang does not even teach that replating the cells will result in at least a 10-fold expansion of the cells, there is no reasonable expectation of success. Nowhere does Huang teach that replating the cells that failed to grow will result in a 10-fold expansion. With regard to the cell line that did grow (LC3), it is unclear from Huang whether the growth was greater than 10-fold, and so there is no reasonable expectation of success to indicate that replating the one cell line that did grow would result in an expansion of at least 10-fold in the LC3 cell line. Therefore, Huang offers no reasonable expectation of success.

Huang also fails to teach or suggest every claim limitation. Huang does not teach replating the cells to result in an expansion of at least 10-fold. Huang also fails to suggest such a step. However, replating the cells to result in an expansion of at least 10-fold is a claim limitation that must be taught or suggested in the prior art reference. Therefore, the Examiner has not met the burden of establishing a *prima facie* case of obviousness.

Huang disclosure regarding the use of conditioned media does nothing to change the fact that the Examiner has not established a *prima facie* case of obviousness. Huang still demonstrates that initially plating marrow stromal cells at a density included in the presently pending claims results in a complete growth failure in one (LC1) of the three cell lines, regardless of the type of conditioned media used. Even in the cell line that performed the best, LC3, Huang only teaches a 3-fold increase in the number of colonies, and as stated above, it is unclear and speculative to assert that this is equal to an at least 10-fold expansion in the number

of cells, as presently claimed. Further, all three cell lines demonstrated dramatically different results when treated with the same compounds. LC1 failed to grow regardless of the type of conditioned media applied. LC2 demonstrated growth results that were always about half to two-thirds of those achieved by LC3. LC3 grew to almost identical numbers regardless of what type of conditioned media was applied. Huang has demonstrated nothing about the effects of cell density or conditioned media, Huang has only demonstrated that there are three different cell lines that grow very differently despite having a common source, being plated at the same densities and being grown in the same conditioned media. A skilled artisan could not take these data and apply them to the present invention because the data in Huang demonstrate that despite basic similarities, such as cell source, initial plating numbers and conditioned media, cells that should be expected to act similarly in fact display remarkably divergent characteristics. No reasonable expectation of success can be derived from the data in Huang, and therefore, a *prima facie* case of obviousness has not been established.

The Examiner argues that Applicants have failed to provide any evidence as to why the skilled artisan would not use the methods taught by Huang to replate and expand the cells. However, it is not the Applicants' burden to establish such evidence. As set forth in MPEP § 2142, the Examiner has the initial burden of factually supporting a *prima facie* case of obviousness, and only after a *prima facie* case has been established does it become Applicants' burden to rebut the Examiner's case with evidence of nonobviousness. As stated in § 2142, “[i]f the examiner does not produce a *prima facie* case, the applicant is under no obligation to submit evidence of nonobviousness.” As established above, the Examiner has not established a *prima facie* case, so it is not the Applicant's duty to supply evidence of nonobviousness, even though Applicants have supplied such data by way of the deficiencies in Huang, stated herein. However, regardless of Applicants' burden, the Examiner has not met the initial burden required by law. The MPEP requires that the Examiner factually support a *prima facie* case of obviousness. The Examiner has not done so. The Examiner has speculated that the skilled artisan would replate cells to arrive at the induced level of proliferation presently claimed, but provides no facts or evidence. Since Huang does not teach replating cells, where is the factual basis for such an assertion? Unless the Examiner can establish such a factual basis for replating the cells, a *prima facie* case of obviousness has not been demonstrated, and the Examiner's

burden has not been met.

For the reasons stated above, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness, and therefore Huang fails to render the present invention unpatentable. Reconsideration and withdrawal of the rejection of claims 1-12, 14-29 and 31-36 is respectfully requested at this time.

Rejection of Claims 1, 22-29 and 31-36 Pursuant to 35 U.S.C. §103(a)

Claims 1, 22-29 and 31-36 stand rejected pursuant to 35 U.S.C. §103(a) as allegedly being rendered obvious by Huang in view of Kuznetsov, et al. (1997, J. Bone and Mineral Res., 12: 1335-1347; hereinafter “Kuznetsov”), Azizi et al., (1998, Proc. Nat’l. Acad. Sci. USA, 95: 3908-3913; hereinafter “Azizi”), Greenberger (U.S. Patent No. 5,766,950); hereinafter “Greenberger”), and Prockop (1997, Science, 276: 71-74; hereinafter “Prockop”). The Examiner contends that the present claims are rendered obvious for the reasons set forth in the Office Action dated August 10, 2004.

The three elements the Examiner is required to establish in order to demonstrate a *prima facie* case of obviousness are discussed above, as are the deficiencies in Huang, making that reference insufficient to render the present claims unpatentable. Kuznetsov, Azizi, Greenberger and Prockop do nothing to correct these deficiencies.

Huang discloses an initial plating density within the scope of the present claims, but does not teach nor suggest replating the cells or replating the cells such that the cells are expanded by a factor of 10-fold. Kuznetsov discloses an initial plating density ranging from 7-14,000 cells per cm² and a replating density that is not specified in terms of cells per cm², but rather in terms of colonies. The only specific replating density referred to is in the first paragraph of the second column page 1337, which is 50,000 cells in the unspecified surface area of a two-well chamber. Further, Kuznetsov does not teach or suggest that replating these cells at any density resulted in an expansion of at least 10-fold. Greenberger initially plates cells at a density of 1 X 10⁸ cells in a T150 flask (666,666.67 cells per cm²) and then splits these cells at 1:2 or 1:3 (see column 6). However, Greenberg does not disclose the number of replated cells per cm² after the split, and therefore does not teach or suggest that less than about 50 cells are replated, or that replating less than about 50 cells will result in an expansion of at least 10-fold. Azizi teaches that 3 X 10⁶ cells are plated in 25 cm² dishes (120,000 cells per cm²) grown to

confluence, and then split at a ratio of 1:2 or 1:3 and replated. Even if the cells did not grow, Azizi teaches replating at a minimum density of 40,000 cells per cm². Assuming the cells did grow, Azizi teaches replating the cells at a density much greater than 40,000 per cm². Azizi does not teach or suggest that replating the cells at any density will result in an expansion of the cells of at least 10-fold. Prockop does not teach inducing the proliferation of marrow stromal cells at any cell density or replating the cells at any cell density such that the cells expand at least 10-fold.

It is therefore irrelevant that Kuznetsov, Greenberger, and Azizi teach the use of growth factors or conditioned medium in growing marrow stromal cells because each reference fails to teach or suggest plating marrow stromal cells at an initial density of less than about 50 cells per cm², and replating the cells at a density of less than about 50 cells per cm² such that the cells expand by at least 10-fold. The skilled artisan cannot have a reasonable expectation of success in arriving at the present invention if these key elements of the claims are not taught or suggested, regardless of the use of growth factors or conditioned media. Further, since none of these key elements are taught in any of the references cited by the Examiner, either alone or in combination, the necessary elements of a case of *prima facie* obviousness have not been met.

For the reasons stated above, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness, and therefore Huang in view of Kuznetsov, Azizi, Greenberger and Prockop fails to render the present invention unpatentable. Reconsideration and withdrawal of the rejection of claims 1, 22-29 and 31-36 is respectfully requested at this time.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is not inapplicable, and that claims 1-12, 14-29 and 31-36 are now in condition for allowance. Applicants further submit that no new matter has been added by way of the present amendment. Reconsideration and allowance of the claims is respectfully requested.

Respectfully submitted,

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Enclosures: Petition for Three Month Extension of Time
Revocation of Power of Attorney and Appointment of New Attorney by Assignee
Notice of Appeal
Petition for Revival of an Unintentionally Abandoned Application

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